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ANALYSIS OF THE HUMAN T CELL RESPONSE TO HTLV-III (AIDS)

Annual Report

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Acquired Immune Deficiency Syndrome is marked by a collapse in immune responses, particularly those mediated by thymus-derived (T) lymphocytes. Nevertheless, the presence of antibodies to the AIDS virus (HIV) indicates the presence of virus specific T cells. In the course of attempting to expand virus specific T cells from HIV seropositive patients, we have determined that the immunomodulator interferon γ (IFN-γ) is capable of containing T cell responses in patients with AIDS. These effects occur for T cells responsive to both mitogens and antigens, but only in moderately advanced (not in far advanced) disease.

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FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR56.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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I. STATEMENT OF THE PROBLEM:

Although patients with HIV infection have depressed T cell function, the fact that they produce antibodies to HIV proteins (1) and even have lymphocytes capable of lysing infected target cells (2) indicates the presence of an immune response to this virus. The purpose of this contract is to devise methods to characterize that T cell response in a variety of patient populations. Once the T cell response has been defined, it should be possible to attempt to augment any response which could be effective at the elimination of virus as well as potentially dampening ineffective responses, eliminating unwanted responses.

II. BACKGROUND AND REVIEW OF APPROPRIATE LITERATURE

In formulating our hypothesis, we relied on the fact that patients with HIV infection have a good antibody response to the viral proteins (1) and the fact that in other human retroviruses (e.g. HTLV-1) cytolytic T cells can be demonstrated (3, 4). Recently Walker and others have demonstrated the presence of lymphocytes with the capacity to lyse infected target cells in the blood of patients with HIV (2). Although previous studies of virus specific T cells had concentrated on surface glycoproteins, recent data indicates that internal proteins may be important in stimulating T cells (5). For these reasons, we are utilizing a variety of proteins as stimuli. The recent use of peptides rather than whole proteins as stimuli for target for CTL (6) has broadened our horizons of potential antigens which can be evaluated.

III. RATIONALE USED IN CURRENT STUDY

Our eventual goal (as stated in the contract proposal) will be to augment protective immunity. Animal stains indicate that this can be done by the development of anti-T cell receptor antibodies (7). In order to obtain such reagents, however, it is necessary to clone T cells. To do this, we have had to develop methods to 1) support the growth of T cells from HIV positive patients and 2) define what antigens will be stimulated.

IV. EXPERIMENTAL METHODS

In order to support the growth of lymphocytes from HIV positive patients, we have used the following protocol:

Isolation of Mononuclear Peripheral Blood Cells and Proliferation Assays:

Human mononuclear peripheral blood cells (PBL) were isolated under sterile conditions from 60-100 ml heparinized peripheral blood by ficoll-hypaque density gradient centrifugation. Thirty ml aliquots of blood were layered over 15 ml of lymphocyte separation (LSM) medium (Litton Bionetics, Kensington, MD). After centrifugation, cells at the interface were collected, and washed twice in a culture medium consisting of RPMI 1640 medium (Gibco, Grand Island, New York) supplemented with 5% human AB serum (MA Bioproducts, Walkersville, MD), 2mM glutamine (Gibco), and 100 U/ml penicillin-streptomycin (Gibco).

To evaluate the effects of different recombinant lymphokines, we have looked initially at responses to microbial antigens.

The PBL were isolated, washed, and resuspended (10^6/ml) in medium as described above. Subsequently, the cells were transferred in 100 ml portions to Falcon 96 well flat-bottom microtiter plates (Becton-Dickinson Labware, Oxnard, CA). The cytokines, PHA (Difco, Detroit, MI), and the microbial antigens, including HSV, CMV, and *Candida albicans* were prepared in culture medium and added to the wells in 50 ml volumes. The soluble antigens were used at the dilution which elicited the optimal lymphoproliferative response in the AIDS-KS cultures.

The lymphocyte cultures were incubated for five days at 37° in 5% CO_2. Tritiated (^{3}H-) thymidine, 50 ml (1mCi), was added and the incubations were continued for an additional 18-24 h. Subsequently, the cultures were harvested on filters with a Skatron MASH cell harvester and counted in a liquid scintillation counter.

Preparations of purified human cytokines derived from recombinant E. coli were used in these studies. Recombinant IFN-γ was supplied by Biogen, Inc. (Cambridge, MA); the rIFN-α was from Hoffman La Roch (Nutley, NJ) and Schering (Kenilworth, NJ). The recombinant IL-2 was purchased from Genzyme. In order to look at the response to HIV specific proteins, (as noted above), we have taken advantage of several recent developments. We have obtained the vaccinia virus recombinant expression gp120 from Dr. Bernard Moss (8).

In addition, we have obtained a series of peptides made from gp120, gp41 as well as TAT. These peptides were obtained from Dr. Richard Doherty and are now available for our use as stimuli.

Statistical Analysis

The effects of IFN-γ and IFN-α on proliferation were evaluated by using the appropriate comparison procedure (Friedman's rank sum test, Newman Keuls' multiple range test, and Wilcoxin's signed rank test). Differences between the KS and OI groups of patients were evaluated by using the student's T test.

RESULTS

The Effects of rIFN-γ and rIFN-α on the Proliferative Responses of Lymphocytes Isolated from Patients with Kaposi's Sarcoma

The *in vitro* addition of IFN-γ but not -α to the PBL isolated from patients with AIDS-KS resulted in an augmented proliferative response to the lectin PHA (P, <0.01; Wilcoxin's Friedman's test) (Fig. 1). The proliferative response to the viral antigens, HSV and CMV, and to *Candida albicans* was increased in the AIDS-KS cultures supplemented with IFN-γ (P, <0.01; Neuman Keuls' test) (Fig. 2, a-c). Interferon-α, on the other hand, inhibited the responsiveness of the AIDS-KS lymphocytes to PHA (P, <0.05; Wilcoxin's and Friedman's test) (Fig. 1) and to the microbial antigens, HSV, CMV< and *Candida albicans* (P, <0.01; Neuman Keuls' test ((Fig. 2, a-c).

Neither rIFN-γ nor rIFN-α were directly mitogenic. In the absence of an additional mitogenic stimulus (lectin or antigen) the cytokines were without effect on the proliferation of the lymphocytes (P, >0.05, NS; Neuman Keuls' test).

The Effects of IFN-γ and IFN-α on the Proliferative Responses of Lymphocytes Isolated from Patients with a History of Opportunistic Infection

Interferon-γ significantly increased the response to PHA in cultures established from the AIDS-OI patients (P, <0.01; Wilcoxin's and Friedman's test) (Fig. 3). Whereas, IFN-γ enhanced the proliferation to HPA of the lymphocytes isolated from the AIDS-OI patients, IFN-α was without effect on the lectin response (P, >0.05, NS; Wilcoxin's and Friedman's test) (Fig. 3).

In the cultures of lymphocytes derived from the AIDS-OI patients neither IFN-γ nor IFN-α were found to have significant effects on the proliferative response to the microbial antigens (P, > 0.05, NS; Neuman Keuls' test) (Fig. 4, a-c). The effects of the cytokines at doses of 5, 500, and 50,000 U/ml were equivalent in the AIDS-OI cultures stimulated with the microbes. Interferon-γ, at doses of 5 to 50,000 U/ml, failed to stimulate the proliferative response to antigens of the lymphocytes isolated from the AIDS-OI patients.

Immune Parameters in the Patient Groups

The magnitude of the proliferative responses to PHA and to the microbial antigens (in the absence of either cytokine) differed significantly between the AIDS-KS and AIDS-OI groups (see Figs. 1-4). The AIDS-KS, compared with the AIDS-OI, patients had the greater response to PHA (P = 0.01; student's t test), to HSV (P = 0.001; student's t test), to CMV (P = 0.01; student's t test), and to *Candida albicans* (P = 0.02; student's t test).

In addition, the percentages of CD3+ and CD4+ lymphocytes, the CD4/CD8 (ratios), as well as, the WBC (cumm), differed significantly between the two patient groups; the AIDS-KS patients had the greater values (P, < 0.01; student's t test) (Table 1). The relative numbers of lymphocytes and CD8+ cells were similar between the two groups (P, > 0.05, NS; Student's t test) (Table 1).

DISCUSSION AND CONCLUSIONS

We investigated the effects of highly purified, recombinant preparations of IFN-γ and -α on the proliferation of cultured lymphocytes from patients with AIDS. Interferon-γ, but not IFN-α, enhanced the response to lectin and specific antigens of lymphocytes isolated from the AIDS-KS patients (Figs. 1 & 2 a-c). Whereas, IFN-γ increased proliferation, IFN-α decreased the lectin and antigen responses of the AIDS-KS cells (Figs. 1 & 2 a-c). Interferon-γ augmented the lectin-stimulated proliferation of lymphocytes harvested from the AIDS-OI patients (Fig.3); but, the lymphokine was without effect in the AIDS-OI cultures stimulated with microbial antigens (Fig. 4 a-c). The incubation of the AIDS-OI lymphocytes with IFN-α had no effect on the response to PHA or to the microbes (Figs. 3 & 4 a-c).

It is noteworthy that the magnitude of the proliferative responses responses to lectin and to the microbial antigens (in the absence of either cytokine) differed significantly between the AIDS-KS and AIDS-OI groups (see Figs. 1-4). The AIDS-KS, compared with the AIDS-OI, patients had the greater response to PHA and to the microbial antigens (in the absence of either cytokine), as well as, the higher lymphocyte values, including the percentages of CD3+ and CD4+ lymphocytes, and the CD4/CD8 ratios (Table 1).

Interferon-γ increased proliferation to the microbial antigens of the lymphocytes isolated from the AIDS-KS, but not, the AIDS-OI patients. Thus, IFN-γ augmented the lymphocyte responsiveness to the antigens in the AIDS patients with the "better" proliferative responses and the higher lymphocyte (CD3+, CD4+, and CD4/CD8) values.

Interferon-γ may increase the proliferative response of cultured lymphocytes from patients with AIDS via its antiviral and/or immunomodulating activity. The lymphokine has been shown to inhibit reverse transcriptase activity and the expression of viral antigens in an HIV infected monocytic cell line. However, IFN-α, a potent antiviral agent which has been shown to inhibit in vitro the replication of HIV (9) failed to increase the proliferative responses of the AIDS lymphocytes. It seems unlikely, therefore, that the effects of IFN-γ were solely the result of its antiviral activity.

Interferon-γ is known to be a stimulus for the expression of Ia molecules on Ac, for the secretion of the monokine IL-1, and for the activation of macrophages. The enhancement of T cell proliferation by IFN-γ is most likely related to the capability of this lymphokine to increase the expression of Ia antigens on the Ac and/or to stimulate the release of IL-1 from monocytes/macrophages. In AIDS certain Ac, including peripheral blood monocytes and Langerhans cells from the skin, exhibit a deficiency in Ia antigens. (10,11) Previously, IFN-γ has been shown to increase the expression of Ia molecules on adherent monocytes (10, 11) harvested from the peripheral blood of patients with AIDS to levels near those of normal cells. The expression of Ia molecules is a strict requirement for the antigen presenting functions associated with Ac which are needed to elicit T helper cell responses to viruses and nominal antigens .28-31

The enhancement of T cell proliferation by IFN-γ may also be related to the capability of this lymphokine to enhance the release of IL-1. The monokine IL-1 plays a crucial role in cellular immune responses as it is required for vital steps in T cell activation. Interleukin-1 has been shown to stimulate the release of IL-2, induce the expression of IL-2R upon activated T cells, and function in the primary activation of T lymphocyte responses to antigens and mitogens. T cell proliferation is dependent upon successful interaction between IL-2 and IL-2R. Interferon-γ enhances the release of IL-1 and since IL-1 is a stimulus for the secretion of IL-2 and the induction of IL-2R, IFN-γ most likely affects proliferation, indirectly, through the elaboration of IL-1 and IL-2.

The IFN-γ secretory defect has been identified in patients with the AIDS related complex (ARC). Thus, a reduction in the level of IFN-γ occurs before the development of full-blown AIDS and in the early stages of the disease. The deficiency in IFN-γ could, at least in part, precipitate a number of the other AIDS related defects, including the diminished release of IL-1 and IL-2, the decreased expression of IL2R on activated T lymphocytes, and the reduced expression of Ia antigens on monocytes and other accessory cells.

In animal models IFN-γ has been shown to increase macrophage oxidative metabolism and promote the killing of certain intracellular pathogens, including *Toxoplasma gondii*, *Leishmania donovani*, *Listeria* monocytogenes, and *Mycobacterium intracellulare*.12-15 Previous studies have shown the addition of IFN-γ to cultures of AIDS monocytes/macrophages resulted in an increase in microbiocidal activity. Murphy et al reported that a group of AIDS patients treated with IFN-γ, as compared with a group treated with IL-2, experienced markedly fewer nonopportunistic bacterial infections. The lymphokine may, therefore, exert an antibacterial effect against nonopportunistic bacteria when administered to patients with full-blown AIDS.

Previously, the addition of IFN-γ, concomitant with other agents including the antiviral drug suramin, to AIDS lymphocytes has been reported to enhance the secretion of IL-2. Observations by Zagury et al have indicated that the secretion of IL-2 in response to lectin activation was restored to near normal levels in some AIDS cultures supplemented with a mixture of IFN-γ, suramin, and an anti- IFN-α antibody. Our studies show IFN-γ capable of stimulating the proliferative response to lectin and to antigens of lymphocytes isolated from AIDS-KS patients. Whereas, IFN-γ augmented the lectin-stimulated proliferation of lymphocytes harvested from the AIDS-OI patients the lymphokine failed to increase the response to the microbial antigens in the AIDS-OI cultures. Other studies have indicated that IFN-γ therapy has had little effect on lymphocyte functions of patients with far-advanced AIDS. Our results suggest that IFN-γ therapy should be considered before the development of OI and in earlier stages of HIV disease. Additional studies are needed to adequately evaluate the therapeutic potential of IFN-γ in the treatment of HIV disease.

Having shown that we can support T cells from HIV positive patients with lymphokines, we will now move to cloning individual T cells using the defined peptides (see above) and vaccinia vectors. These experiments are ongoing (as originally proposed for Year II in the submission).

FIGURE LEGEND

Fig 1. The Effects of IFN-γ and IFN-α on the response to PHA of peripheral blood lymphocytes isolated from AIDS patients with Kaposi' sarcoma. The responses to PHA are plotted (mantissa) vs. the responses to PHA + IFN-γ ( ) and to PHA + IFN-α ( ) on the abscissa. The lymphocytes (1×10^5) were activated with PHA (10 mg/ml,final conc.) and selected cultures received either IFN-γ or IFN-α (500 U/ml,final conc.). The cultures were incubated 5 days, ^{3}H-thymidine (1 miCi) added, and the incubations continued an additional 18 h. Proliferation was measured as ^{3}H-thymidine uptake; the data are expressed as the mean values (cpm) for 4 replicate cultures.

Fig 2. The Effects of IFN-γ and IFN-α on the response to microbial antigens of peripheral blood lymphocytes isolated from AIDS patients with Kaposi' sarcoma. The responses to the antigens are plotted on the mantissa vs. the responses to the microbes + IFN-γ ( ) and + IFN-α ( ) on the abscissa. The lymphocytes (1×10^5) were stimulated with the microbes or with the microbes + the cytokines (500 U/ml,final conc.). The microbial antigens were inactivated before addition to the cultures; HSV and CMV were exposed to UV irradiation and *Candida albicans* was incubated at 55o C for 60 min. The cultures were incubated 5 days, ^{3}H-thymidine (1 miCi) added, and the incubations continued an additional 24 h. Proliferation was measured as ^{3}H-thymidine uptake; the data are expressed as the mean values (cpm) for 4 replicate cultures. (Panel A, CMV; Panel B, HSV; Panel C, Candida).

Fig 3. The Effects of IFN-γ and IFN-α on the response to PHA of peripheral blood lymphocytes isolated from AIDS patients with prior opportunistic infections. Culture conditions were as described for Fig.1. Proliferation was measured as ^{3}H-thymidine uptake. The data are presented as described in Fig. 1.

Fig 4. The Effects of IFN-γ and IFN-α on the response to microbial antigens of peripheral blood lymphocytes isolated from AIDS Patients with prior opportunistic infection. Culture conditions were as described for Fig. 2. Proliferation was measured as ^{3}H-thymidine uptake. The data are presented as described in Fig. 2 (Panel A, CMV; Panel B, HSV; Panel C, Candida).

Table 1. Lymphocyte Values

Patient Groups						
I. Kaposi's Sarcoma (KS)	WBC[a]	L[b]	CD3+[c]	CD4+	CD8+	CD4/CD8
mean	4.2	34	70.4	21.4	36.9	0.63
range	2.8 - 7.3	22 - 51	57 - 89	6 - 38	23 - 50	0.1 - 0.6
II. Previous Opportunistic Infection						
mean	3.4	33	57.7	5.3	46.6	0.13
range	1.4 - 6.0	16 - 67	23 - 80	0.9 - 12.8	23 - 74	0.02 - 0.34
	$p<0.01$	$p>0.05$	$p<0.01$	$p<0.01$	$p>0.05$	$p<0.01$

[a] WBC = White blood cells

[b] L = lymphocytes

[c] The percentages of CD3+, CD4+, and CD8+ lymphocytes were determined by indirect immunofluorescent staining and flow cytometry. The OKT3, OKT4, and OKT8 mouse anti-human monoclonal antibodies were employed to label the cells. Indirect staining of the monoclonal antibodies was accomplished by use of goat F(ab')2 anti-mouse Ig-FITC (Meloy labortories, Springfield, Va.). Antibody staining of the surface antigens was quantitatively analyzed on an Epics II Coulter flow cytometer; 10,000 cells were analyzed.

FIGURE 1

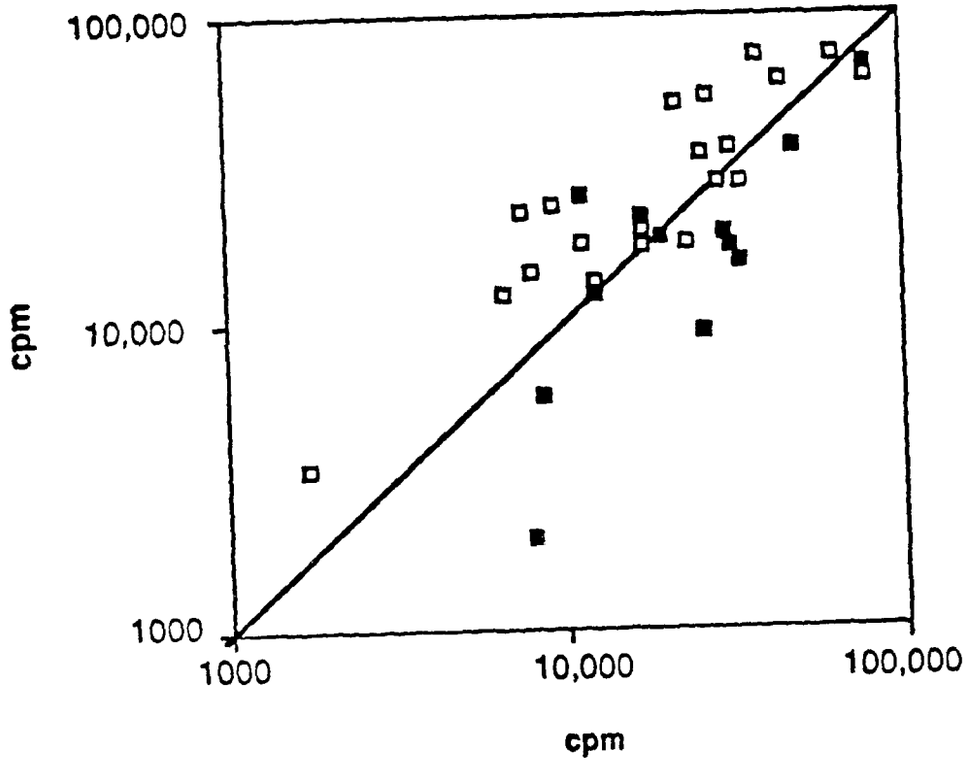

Fig. 1

FIGURE 2

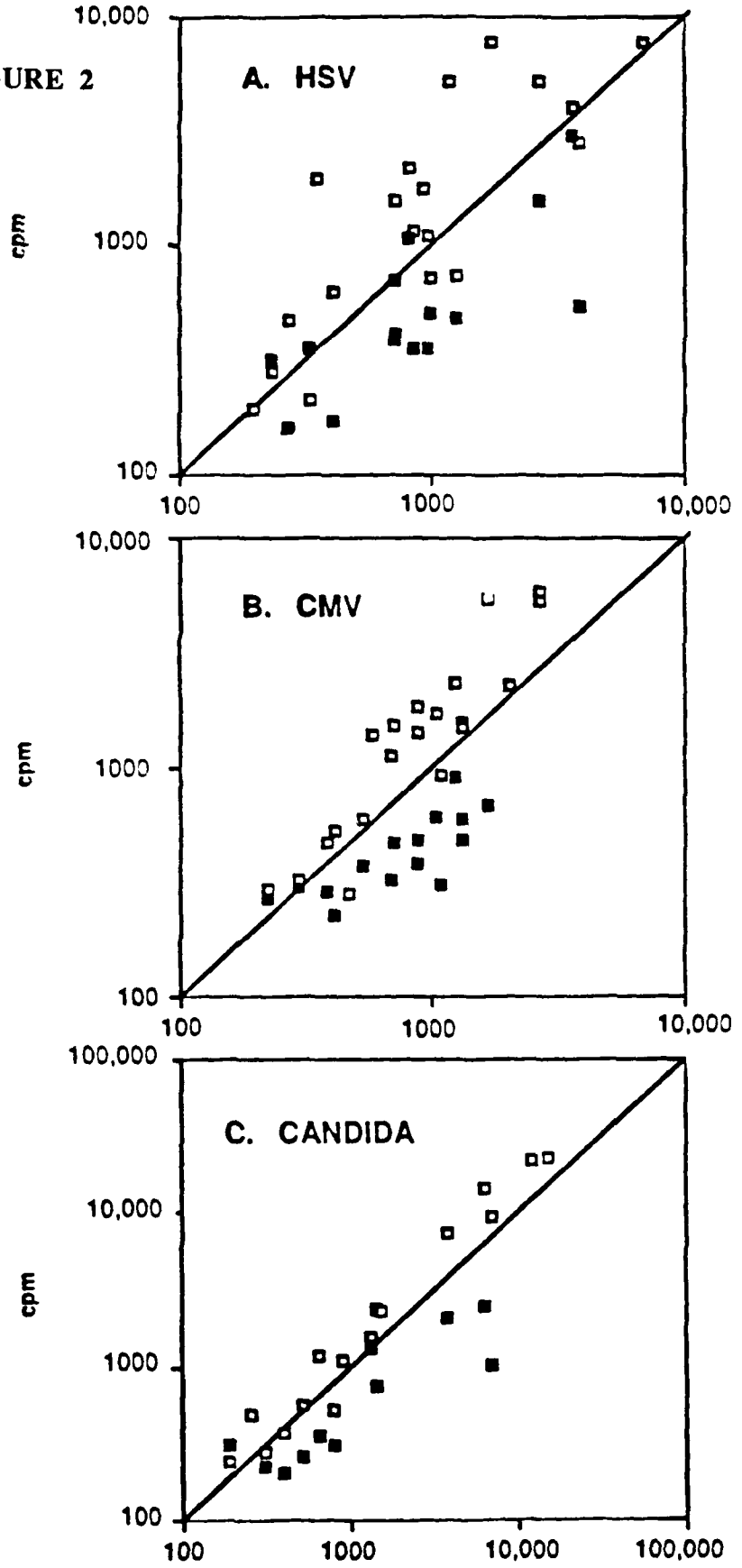

Fig. 2

FIGURE 3

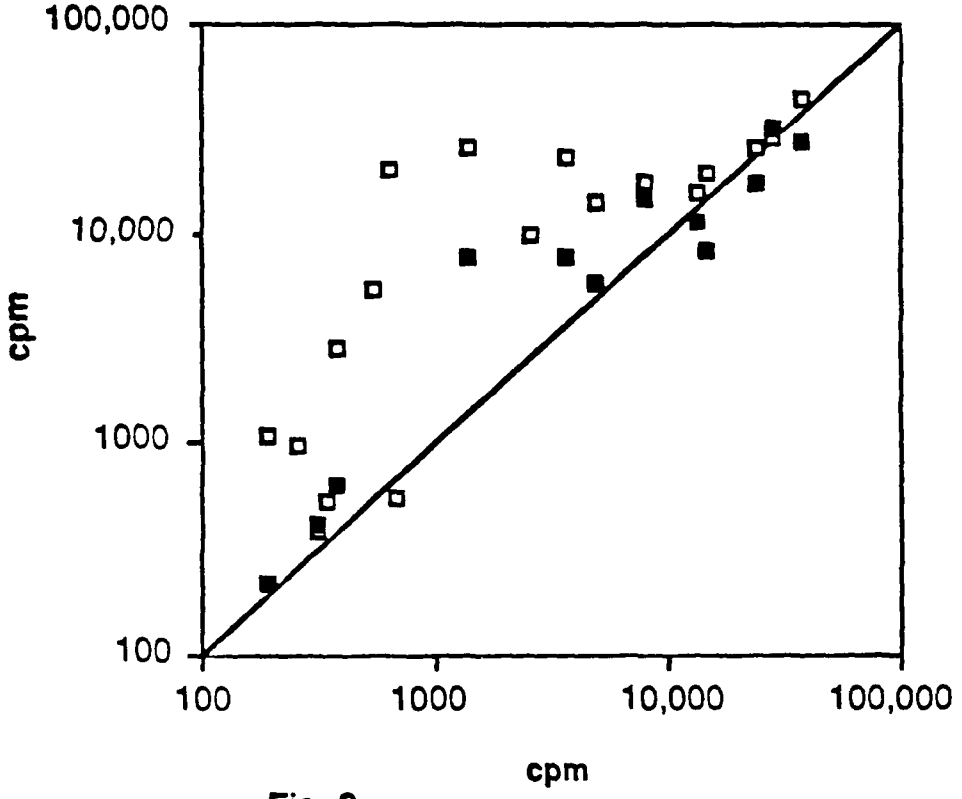

Fig. 3

FIGURE 4

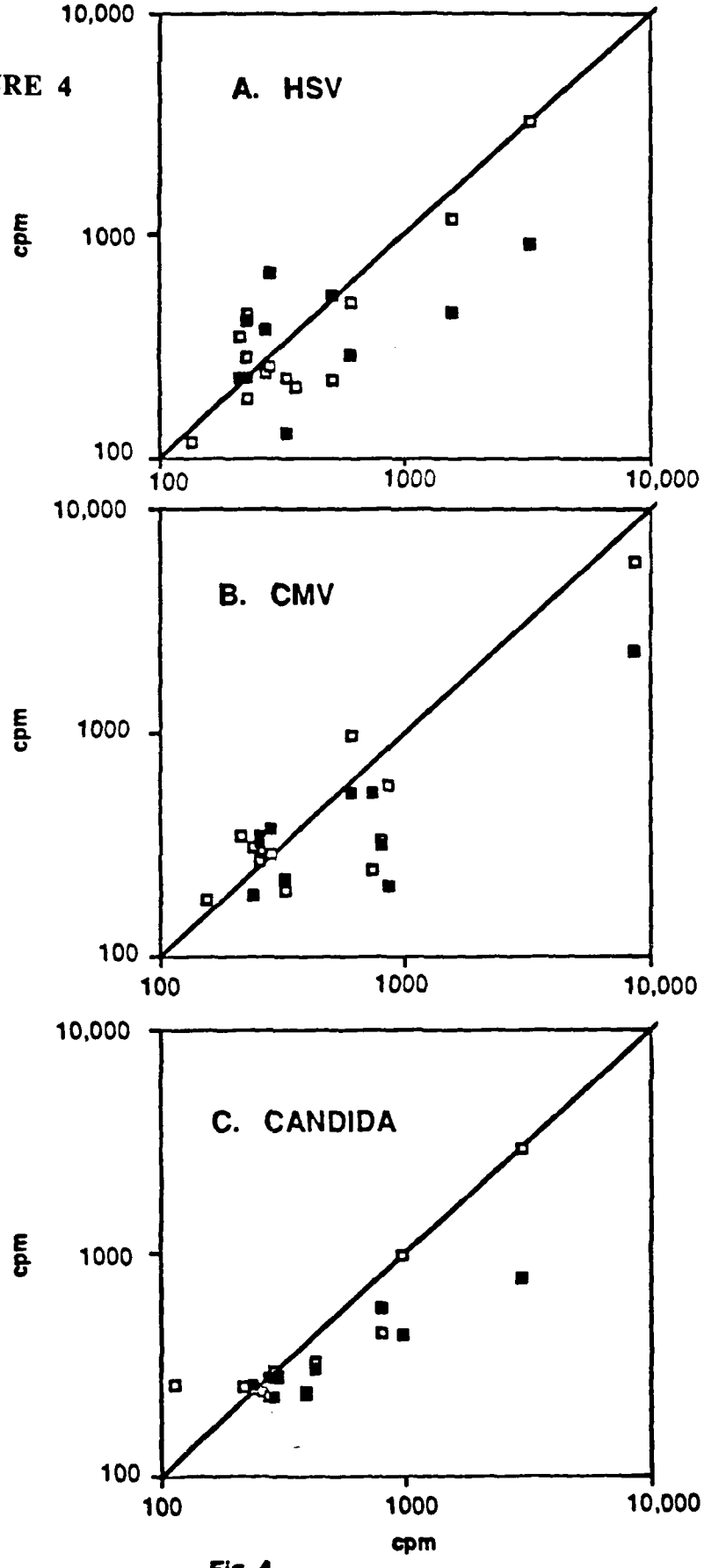

Fig. 4

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